

# The Effect of an Experimental Chlorate Product on *Salmonella* Recovery of Turkeys when Administered Prior to Feed and Water Withdrawal

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**ABSTRACT** Previously, an experimental chlorate product (ECP) has been observed to reduce *Escherichia coli* and *Salmonella* infections in swine, cattle, and broilers. The following studies were performed to investigate the effects of different concentrations and durations of administering ECP on crop and ceca *Salmonella typhimurium* (ST) colonization of turkeys. In 2 separate trials, each conducted with 2 replicates, 15-wk-old turkey toms were challenged with  $10^7$  to  $10^9$  cfu of ST. In Experiment 1, toms were administered 0, 0.5, 1.0, 2.0, or 4.0× of ECP (a 1.0× concentration is equivalent to a 15 mM chlorate ion concentration) in the drinking water for 38 h. In Experiment 2, toms were administered a 2× concentration of ECP in the drinking water for 0, 14, 26, or 38 h prior to water withdrawal. All treatments were followed by a 10-

h water withdrawal and an 8-h feed withdrawal prior to organ sampling. In Experiment 1, turkeys provided ECP had significantly ( $P < 0.05$ ) lower populations and incidences of crop ( $>1.4$  log reduction) and ceca ( $>0.6$  log reduction) ST as compared with control birds (2.1 and 0.94 log ST average for all trials, respectively), with little or no additional benefit from administration of higher ECP concentrations. In Experiment 2, toms provided ECP had lower populations of crop ( $>2.2$  log reduction) and ceca ( $>1.5$  log reduction) ST when compared with controls (3.1 and 1.8 log ST, respectively). Again, there appeared to be little benefit in longer administration intervals on quantitative reduction of ST. These experiments suggest that the ECP significantly reduces *Salmonella* colonization in commercial turkeys when administered prior to feed and water withdrawal.

**Key words:** turkey, experimental chlorate-based product, *Salmonella typhimurium*

2006 Poultry Science 85:2101–2105

## INTRODUCTION

Foodborne illness causes an estimated 76 million illnesses and 5,000 deaths annually in the United States (Mead et al., 1999). Over 200 known diseases have been transported through food, including viruses, parasites, toxins, metals, and bacteria (Bryan et al., 1968). *Salmonella* is the most frequently isolated bacteria from foodborne sources, with poultry and poultry products reported as one of the prevailing vehicles for this bacterium (Bean and Griffin, 1990; Persson and Jendteg, 1992). A number of technologies have been developed to reduce carcass contamination by foodborne pathogens during and after slaughter, including use of vaccines, competitive exclusion products, and chemical alteration of microbial environment (Nurmi and Rantala, 1973; Goodnough and Johnson, 1991; Corrier et al., 1994; Moore and Miller, 1994; Muirhead, 1994; Schoeni and Wong, 1994; Huff et al.,

1996; Moore et al., 1996; Hassan and Curtiss, 1997; Nisbet et al., 1998; Terzich et al., 1998; Zhang-Barber et al., 1999; Sydenham et al., 2000; Byrd et al., 2001), but none are completely dependable, as product recalls and outbreaks of human foodborne disease continue to occur. Therefore, it is imperative that foodborne pathogens are reduced on the farm, because quantitative risk assessments have indicated that such interventions applied just prior to slaughter would reduce human exposure to pathogens (Vugia et al., 2003).

Recently, our laboratory investigated a novel approach that uses a specific intracellular bacterial metabolic pathway to reduce the number of foodborne pathogens in food-producing animals. This pathway found in many *Enterobacteriaceae*, including *Escherichia coli* and *Salmonella*, can metabolize nitrate using a dissimilatory respiratory nitrate reductase enzyme, which, coincidentally, converts inorganic chlorate to cytotoxic chlorite (Pichinoty and Piéchaud, 1968; Brenner, 1984; Gennis and Stewart, 1996). By adding chlorate in the system, the bacteria possessing the nitrate reductase enzyme will intracellularly build up toxic concentrations of chlorite and eventually die. Due to the fact that most strict anaerobic gastrointestinal bacteria lack respiratory nitrate reductase activity

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Received November 22, 2005.

Accepted March 23, 2006.

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(Stewart, 1988), it was demonstrated that beneficial anaerobes lacking the enzyme (Anderson et al., 2000) would not be affected, preserving the competitive exclusion potential of the host's normal flora. The selective bactericidal activity of an experimental chlorate-based product (ECP) was evaluated in both ruminants (Anderson et al., 2002; Callaway et al., 2002; Edrington et al., 2003) and monogastrics (Anderson et al., 2001a,b; Byrd et al., 2003; Jung et al., 2003), supporting the practical use of the product to reduce gastrointestinal concentrations of foodborne pathogens.

Little work has addressed the contamination of turkey carcasses with salmonellae. In one study, Cox et al. (2000) found that 31.1% of turkey toms were positive for *Salmonella* prior to harvest. In the processing plant, *Salmonella* was isolated on 27% of the finished turkey products (Bryan et al., 1968). A logical approach to decrease foodborne pathogens on the final product would be to reduce the number of pathogens entering the processing plant, thus allowing postharvest intervention opportunities to be more effective. Because of the success of ECP reducing *Salmonella* in broilers (Byrd et al., 2003; Jung et al., 2003), we performed the present study with ECP to determine its efficacy in the reduction of *Salmonella* colonization of market turkeys.

## MATERIALS AND METHODS

### Experimental Design

Two experiments were performed to investigate the effect of concentration and duration of the ECP on crop and ceca *Salmonella* recovery.

**Experiment 1.** Two trials were conducted to investigate the effect of concentration of the ECP on crop and ceca *Salmonella* recovery. In each trial, 15-wk-old turkey toms were obtained from a commercial turkey operation, randomly divided into 4 groups of 20 birds per group, and placed in floor pens that contained new pine shavings. Birds were allowed free access to water or water treatments and an unmedicated corn-soybean-based diet, which met or exceeded NRC requirements (National Research Council, 1994). At 5 d and 1 d prior to organ sampling, birds were challenged with  $10^7$  to  $10^8$  cfu of novobiocin (NO)- and nalidixic acid (NA)-resistant *Salmonella typhimurium* (ST). Birds were provided either distilled water with the drug carrier or 0.5, 1.0, or 2.0× of the ECP (a 1.0× concentration is equivalent to a 15 mM chlorate ion concentration) as the drinking water for 38 h in trial 1 and either distilled water with the drug carrier or 1.0, 2.0, or 4.0× ECP added as the drinking water for 38 h in trial 2. Fluid consumption was recorded for the treatment period. Treatment was followed by a 10-h water withdrawal and an 8-h feed withdrawal prior to termination of the experiment. During the 8-h feed withdrawal time, the litter was covered and pens were covered with a tarp to simulate the unavailability of litter and the confinement associated with transport.

**Experiment 2.** Two trials were conducted to investigate the effect of duration of the ECP on crop and ceca *Salmonella* recovery. In each trial, 15-wk-old turkey toms were obtained from a commercial turkey operation and randomly divided into 4 groups of 20 birds per group and placed in floor pens that contained new pine shavings. Birds were allowed free access to water or water treatments and an unmedicated corn-soybean-based diet, which met or exceeded NRC requirements (National Research Council, 1994). At 5 d and 1 d prior to organ sampling, birds were challenged with  $10^8$  to  $10^9$  cfu of NO- and NA-resistant ST. Birds were provided either distilled water alone or distilled water followed by 2.0× ECP (30 mM chlorate ion concentration) in the drinking water for 14, 26, or 38 h, for a total treatment period of 38 h. Fluid and feed (trial 2 only) consumption was recorded for the treatment period. Treatment was followed by a 10-h water withdrawal and an 8-h feed withdrawal prior to termination of the experiment. During the 8-h feed withdrawal time, the litter was covered and pens were covered with a tarp to simulate the unavailability of litter and confinement associated with transport.

### *Salmonella* Inoculation Procedures

*Salmonella* oral gavage challenge inoculates for Experiments 1 and 2 were prepared from a primary poultry isolate of ST obtained from the National Veterinary Services Laboratory (Ames, IA). The isolate was selected for resistance to NO and NA and was maintained in media containing 25 µg/mL of NO (Sigma Chemical Co., St. Louis, MO) and 20 µg/mL of NA (Sigma Chemical Co.). Challenge inocula were prepared from an overnight soy broth (Becton, Dickinson and Company, Sparks, MD) culture serially diluted in sterile PBS. The optical density of the cell dilution was measured with a spectrophotometer (Spec 20D, Milton Roy, Analytical Products Division, Rochester, NY) at 625 nm, and the number of cells for each inoculum was determined using a standard curve. The viable cell concentration of the challenge inocula was confirmed by colony counts on NO and NA brilliant green agar (BGA) plates.

### *Salmonella* Culture Procedures

At termination of each experimental trial, crops and ceca were aseptically collected from all birds. Ceca from each bird were collected into 2 separate sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI). A 0.25-g sample of contents from 1 cecum was collected and serially diluted to a final dilution of 1:10, 1:100, and 1:1,000, and 1 mL and 0.1 mL of the 1:10 dilution and 0.1 mL of the 1:100 and 1:1,000 dilutions were spread plated on NO and NA BGA plates. Crops were placed in sterile Whirl-Pak filter bags (Nasco), and crop weights were recorded. Twenty milliliters of Butterfield's buffer (Sigma Chemical Co.) was added to each bag, and the sample was stomached for 30 s (Stomacher 80, laboratory blender, Tekmar, Cincinnati, OH). One milliliter of stomached crop contents

**Table 1.** Effect of an experimental chlorate compound (ECP) provided in the drinking water for 38 h on *Salmonella typhimurium* (ST) crop and cecal colonization in 15-wk-old turkeys in Experiment 1 (2 trials)<sup>1,2</sup>

	Fluid consumed (mL/bird per h)	Crop <i>Salmonella</i> incidence <sup>3</sup> (+/-)	Crop <i>Salmonella</i> (log <sub>10</sub> ST/g of crop contents)	Ceca <i>Salmonella</i> incidence <sup>3</sup> (+/-)	Ceca <i>Salmonella</i> (log <sub>10</sub> ST/g of ceca contents)
Trial 1					
Control	57.4	13/20 (65%)	2.01 ± 0.40 <sup>a</sup>	7/20 (35%)	0.90 ± 0.31 <sup>a</sup>
0.5× ECP	72.1	5/20 (25%)*	0.59 ± 0.24 <sup>b</sup>	2/20 (10%)	0.27 ± 0.19 <sup>b</sup>
1.0× ECP	58.4	4/20 (20%)*	0.54 ± 0.27 <sup>b</sup>	0/20 (0%)**	0.00 ± 0.00 <sup>b</sup>
2.0× ECP	64.5	0/20 (0%)**	0.00 ± 0.00 <sup>b</sup>	1/20 (5%)*	0.08 ± 0.08 <sup>b</sup>
Trial 2					
Control	47.6	12/20 (60%)	2.20 ± 0.46 <sup>a</sup>	15/20 (75%)	0.99 ± 0.32 <sup>a</sup>
1.0× ECP	66.3	0/20 (0%)**	0.00 ± 0.00 <sup>b</sup>	6/20 (30%)**	0.03 ± 0.01 <sup>b</sup>
2.0× ECP	49.2	0/20 (0%)**	0.00 ± 0.00 <sup>b</sup>	8/20 (40%)*	0.23 ± 0.15 <sup>b</sup>
4.0× ECP	53.7	1/20 (5%)**	0.14 ± 0.13 <sup>b</sup>	7/20 (35%)*	0.04 ± 0.01 <sup>b</sup>

<sup>a,b</sup>Mean values within the same column with no common superscripts differ significantly ( $P \leq 0.05$ ).

<sup>1</sup>1.0× ECP is equivalent to a 15 mM chlorate ion concentration.

<sup>2</sup>Turkeys had no access to water during the last 10 h or feed during the last 8 h of the study to simulate commercial feed withdrawal conditions.

<sup>3</sup>+/- = positive *Salmonella* samples/total samples. A significant difference was found between the number of positive controls and positive, treated crops or ceca (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ).

was serially diluted in tubes containing 9 mL of Butterfield's to obtain 1:10 and 1:100 dilutions of the diluted crop contents, 1 mL and 0.1 mL of the stomached crop contents, and 0.1 mL of the crop contents. Each of these dilutions (a total of 4 dilutions) was spread plated on NO and NA BGA plates for *Salmonella*. An average weight of 10 empty crops was made, crop content volume was estimated by subtracting the average crop weight from individual crop weights, and final crop dilutions were calculated from individual crop content weights. All plates were incubated for 24 h at 37°C, and the number of colony-forming units of *Salmonella* was enumerated and expressed as log<sub>10</sub> *Salmonella* colony-forming units per gram of crop or contents.

Additionally, in trial 2 of Experiment 1 and in both trials of Experiment 2, 50 mL of tetrathionate broth (Difco Laboratories, Detroit, MI) was added to each bag containing the unsampled cecum from each bird, and the bag was stomached for 30 s (Tekmar). In Experiment 2, 20 mL of 2× tetrathionate broth (Difco Laboratories) was added to each bag containing the stomached crop. Tetra-thionate-enriched crop and cecal samples were incubated for 24 h at 37°C and isolated onto NO and NA BGA, which were incubated an additional 24 h for *Salmonella* incidence identification. A subset of each of the positive samples from each trial were confirmed as *Salmonella* with *Salmonella* O (Group B) antisera (Difco Laboratories).

### Statistical Analysis

*Salmonella* colony-forming units were logarithmically transformed prior to analysis to achieve homogeneity of variance and were expressed as log<sub>10</sub> colony-forming units. Differences among treatment groups in crop and ceca counts of *Salmonella* were determined by 1-way ANOVA using the GLM procedure of SAS software (SAS Institute, 1987). Means for each treatment showing significant differences ( $P < 0.05$ ) in the ANOVA were further

separated using Duncan's multiple range test in SAS. Differences among treatment groups in the incidence of *Salmonella* crop and ceca contamination were analyzed by  $\chi^2$  analysis ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

In Experiment 1, 15-wk-old turkey toms administered ECP in the drinking water for 38 h had significantly reduced crop *Salmonella* incidence and number of recoverable *Salmonella* colony-forming units (Table 1). Ceca incidence and recoverable colony-forming units of *Salmonella* was significantly reduced in birds administered 1.0, 2.0, or 4.0× ECP in the drinking water for 38 h (Table 1).

In Experiment 2, turkeys administered 2.0× ECP for 14, 26, or 38 h had significantly reduced crop and ceca incidence of *Salmonella* and number of recoverable *Salmonella* colony-forming units, with the exception of incidence of *Salmonella* in enriched ceca samples in trial 1, which were not significantly reduced for any duration of ECP investigated (Table 2).

Recent research has demonstrated that the bactericidal effect of chlorate treatment is enhanced nearly 100-fold or more in broilers (Jung et al., 2003) and pigs (Anderson et al., 2004) if preceded by a short nitrate preadaptation period, which effectively increases the susceptibility of the target bacteria to chlorate via induction of respiratory nitrate reductase activity.

The use of ECP in a preharvest program needs further investigation to evaluate toxicity, assess pathogen resistance, and maximize efficacy in the field. Previously, chlorate has been used for pathogen reduction in low concentrations in veterinary and human medicine and has been approved for toothpaste products in Europe (Cosmetic Ingredient Review Panel, 1995).

The toxicity of chlorate has been reported to be approximately 800 mg of chlorate ion/kg per day in rats treated with chlorate in the drinking water for 90 d (McCauley



**Table 2.** Effect of a 2× experimental chlorate compound (ECP) provided in the drinking water for 14, 26, or 38 h on *Salmonella typhimurium* (ST) crop and cecal colonization in 15-wk-old turkeys in Experiment 2 (2 trials)<sup>1,2</sup>

	Fluid consumed (mL/bird per h)	Feed consumed (g/bird per h)	Duration of consumption water/feed (h)	Crop <i>Salmonella</i> incidence <sup>3</sup> (+/-)	Crop <i>Salmonella</i> (log <sub>10</sub> ST/g of crop contents)	Ceca <i>Salmonella</i> incidence <sup>3</sup> (+/-)	Ceca <i>Salmonella</i> (log <sub>10</sub> ST/g of ceca contents)
Trial 1							
Control	45.5		38/36	19/20 (95%)	2.94 ± 0.36 <sup>a</sup>	17/20 (85%)	1.85 ± 0.31 <sup>a</sup>
ECP (h)							
14	38.1		14/12	5/20 (25%)**	0.70 ± 0.2 <sup>b</sup>	15/20 (75%)	0.30 ± 0.17 <sup>b</sup>
26	61.5		26/24	4/20 (20%)**	0.41 ± 0.19 <sup>b</sup>	14/20 (70%)	0.34 ± 0.18 <sup>b</sup>
38	60.5		38/36	3/20 (15%)**	0.14 ± 0.13 <sup>b</sup>	14/20 (70%)	0.19 ± 0.13 <sup>b</sup>
Trial 2							
Control	43.0	19.8	38/36	19/20 (95%)	3.27 ± 0.24 <sup>a</sup>	18/20 (90%)	1.82 ± 0.32 <sup>a</sup>
ECP (h)							
14	35.6	14.3	14/12	7/20 (35%)*	0.04 ± 0.01 <sup>b</sup>	4/20 (20%)**	0.13 ± 0.09 <sup>b</sup>
26	39.0	16.8	26/24	8/20 (40%)*	0.13 ± 0.08 <sup>b</sup>	10/20 (50%)*	0.05 ± 0.01 <sup>b</sup>
38	44.1	17.5	38/36	4/20 (20%)**	0.38 ± 0.23 <sup>b</sup>	8/20 (40%)*	0.22 ± 0.10 <sup>b</sup>

<sup>a,b</sup>Mean values within the same column with no common superscripts differ significantly ( $P \leq 0.05$ ).

<sup>1</sup>1.0× ECP is equivalent to a 15 mM chlorate ion concentration.

<sup>2</sup>Turkeys had no access to water during the last 10 h or feed during the last 8 h of the study to simulate commercial feed withdrawal conditions.

<sup>3</sup>+/- = positive *Salmonella* samples/total samples. A significant difference was found between the number of positive controls and positive, treated crops or ceca (\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ).

et al., 1995), and the 50% lethal dose in humans is believed to exceed 1 g/kg (Cosmetic Ingredient Review Panel, 1995). Although BW were not measured for birds in this study, we can estimate that turkeys received less than 320 mg/kg of chlorate ion in the 2.0× dose of ECP (assuming turkeys weighed more than 16 kg) and perhaps less than 250 mg/kg, depending on the actual weight of the toms.

The effectiveness of chlorate administration is efficacious in decreasing *E. coli* O157:H7 and ST in cattle, pigs, and broilers when administered in the drinking water prior to harvest (Anderson et al., 2001b, 2002; Callaway et al., 2002; Byrd et al., 2003; Jung et al., 2003). These studies also have demonstrated that, whereas chlorate significantly reduces the number of pathogens that possess the nitrate reductase enzyme, total culturable anaerobic bacterial numbers are not significantly altered by chlorate treatment (Anderson et al., 2000). Other studies have demonstrated that *E. coli* or *Salmonella* can become resistant to chlorate when grown as a pure culture; however, resistance is unlikely to occur within mixed bacterial populations, because resistant bacteria could not maintain themselves in competitive environments (Anderson et al., 2001b; Callaway et al., 2001).

In summary, results from this study indicate that novel experimental preparations containing chlorate may have applications in the preharvest control of enteric pathogens. This study further suggests that ECP may be a viable strategy in the reduction of gastrointestinal pathogens prior to harvest and will thereby reduce the amount of cross-contamination of carcasses in the processing plant.

## ACKNOWLEDGMENTS

We thank Clayton Myers, Earl Munson, and Maurice Connell of USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX for their excellent technical assistance.

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